# Viral RNA / DNA isolation

## User manual

NucleoMag® 96 Virus

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MACHEREY-NAGEL
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# 1 Components

## 1.1 Kit contents

| | NucleoMag® 96 Virus | |
|---|---|---|
| | **1x 96 preps** | **4 x 96 preps** |
| **REF** | **744800.1** | **744800.4** |
| NucleoMag® V-Beads | 3 mL | 4 x 3 mL |
| Lysis Buffer MVL | 20 mL | 80 mL |
| Binding Buffer MV2 | 60 mL | 2 x 120 mL |
| Wash Buffer MV3 | 50 mL | 2 x 100 mL |
| Wash Buffer MV4 | 50 mL | 2 x 100 mL |
| Wash Buffer MV5 | 55 mL | 2 x 110 mL |
| Elution Buffer MV6 | 10 mL | 40 mL |
| Carrier RNA* | 400 µg | 4 x 400 µg |
| Carrier RNA Buffer | 500 µL | 4 x 500 µL |
| Proteinase K (lyophilized)* | 22 mg | 4 x 22 mg |
| Proteinase Buffer PB | 3.6 mL | 8 mL |
| User manual | 1 | 1 |

* For preparation of working solutions and storage conditions see section 3.

## 1.2 Material to be supplied by user

| Product | REF | Pack of |
|---|---|---|
| • **Separation plate for magnetic beads separation,**<br>e.g., Square-well Block (96-well block with 2.1 mL square-wells) | 740481<br>740481.24 | 4<br>24 |
| • **Lysis tubes for incubation of samples and lysis,**<br>e.g., Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips) | 740477<br>740477.24 | 4 sets<br>24 sets |
| • **Elution plate for collecting purified nucleic acids**,<br>e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)<br>e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 300 µL flat-bottom wells) | 740486.24<br>740673 | 24<br>20 |
| **For use of kit on KingFisher® 96 instrument:** | | |
| e.g., KingFisher® 96 Accessory Kit A (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® 96 Virus preps using KingFisher® 96 platform) | 744950 | 1 set |

# 2 Product description

## 2.1 The basic principle

The **NucleoMag® 96 Virus** kit is designed for the isolation of viral DNA or RNA from cell-free body fluids such as serum or plasma. This kit provides reagents and magnetic beads for isolation of 96 samples from 200 µL serum or plasma. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with a new and improved Lysis Buffer MVL containing chaotropic ions supported by Proteinase K digestion. For binding of nucleic acids to the paramagnetic beads, Binding Buffer MV2 and the NucleoMag® V-Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers MV3 and MV4. Residual ethanol from previous wash steps is removed by a short incubation of the beads in Wash Buffer MV5. Finally, highly pure viral RNA/DNA is eluted with low-salt Elution Buffer MV6 or water. Purified viral RNA/DNA can directly be used for downstream applications. The **NucleoMag® 96 Virus** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

## 2.2 Kit specifications

**NucleoMag® 96 Virus** is designed for rapid manual and automated small-scale preparation of viral RNA/DNA from cell-free body fluids such as serum or plasma samples. The kit is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information) or other magnetic separation systems (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The purified RNA/DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

**NucleoMag® 96 Virus** allows easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag® SEP on the automation platform.

## 2.3 Magnetic separation systems

For use of **NucleoMag® 96 Virus,** the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information). The kit can also be used with other common separators.

| Magnetic separator | Separation plate or tube |
|---|---|
| NucleoMag® SEP (MN REF 744900) | Square-well Block (MN REF 740481) |
| Tecan Te-MagS™ | 1.5 mL tubes without lid (Sarstedt) |

**Static magnetic pins**

Separators with static magnetic pins, for example, NucleoMag® SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

**Movable magnetic systems**

Separators with moving magnetic pins: Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

**Automated separators**

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

## 2.4 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

**Adjusting shaker speed for binding and wash steps:**

- Load 1000 µL (for checking the settings for the binding step) or 600 µL (for checking the settings for the washing steps) dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing step.

**Adjusting shaker speed for the elution step:**

- Load 100 µL dyed water to the wells of the collection plate and proceed as described above.

## 2.5 Handling of beads

**Distribution of beads**

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads/binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

**Magnetic separation time**

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended using the separation plates or tubes specified by the supplier of the magnetic separator.

**Washing the beads**

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing

of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

| Method | Resuspension efficiency | Speed | Number of tips needed |
|---|---|---|---|
| Magnetic mix | + | ++ | Low |
| Shaker | ++ | ++ | Low |
| Pipetting | +++ | +* | High |

## 2.6 Elution procedures

Purified viral RNA/DNA can be eluted directly with the supplied Elution Buffer MV6. Elution can be carried out in a volume of ≥ 50 µL. It is essential to cover the NucleoMag® Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, high elution volumes might be necessary to cover the whole pellet.

* 8-channel pipetting device

# 3 Storage conditions and preparation of working solutions

**Attention:**

*Buffers MVL, MV2, and MV3 contain chaotropic salt! Wear gloves and goggles!*

- All components of the **NucleoMag® 96 Virus** kit should be stored at room temperature (18–25 °C) and are stable for up to one year.
- All buffers are delivered ready-to-use.
- **Lysis Buffer MVL**:

  Lysis Buffer MVL may form salt precipitates upon storage. To re-dissolve the salt precipitate incubate the buffer bottle at 40 °C until all of the precipitate is re-dissolved.
  Lysis Buffer MVL with Carrier RNA: Lysis Buffer MVL with added Carrier RNA can be stored at room temperature for 1–2 weeks.

  *Frequent warming, temperatures > 80 °C, and extended heat incubation will cause degradation of the Carrier RNA. This leads to reduced recovery of viral RNA and eventually false negative RT-PCR results, in particular, if low-titer samples are used. Do not warm Buffer MVL containing Carrier RNA more than 6 times!*

Before starting any **NucleoMag® 96 Virus** protocol, prepare the following:

- **Proteinase K:** Before first use of the kit, add 1.1 mL Proteinase Buffer PB to each vial of the **lyophilized Proteinase K**. Dissolved Proteinase K solution should be stored in aliquots at -20 °C.
- **Carrier RNA:** Before first use of the kit, add 500 µL Carrier RNA Buffer to each vial **lyophilized Carrier RNA**. Store dissolved Carrier RNA solution in aliquots at -20 °C.

| | NucleoMag® 96 Virus | |
|---|---|---|
| | **1 x 96 preps** | **4 x 96 preps** |
| **REF** | **744800.1** | **744800.4** |
| Proteinase K (lyophilized) | 1 vial (22 mg) | 4 vials (22 mg/vial) |
| | Add 1.1 mL Proteinase Buffer | Add 1.1 mL Proteinase Buffer to each vial |
| Carrier RNA (lyophilized) | 1 vial (400 µg) | 4 vials (400 µg/vial) |
| | Add 500 µL Carrier RNA Buffer | Add 500 µL Carrier RNA Buffer to each vial |

# 4 Safety instructions

The following components of the **NucleoMag® 96 Virus** kits contain hazardous contents.

*Wear gloves and goggles and follow the safety instructions given in this section.*

## 4.1 Risk and safety phrases

| Component<br>*Inhalt* | Hazard contents<br>*Gefahrstoff* | Hazard symbol<br>*Gefahrstoff-symbol* | Risk phrases<br>*R-Sätze* | Safety phrases<br>*S-Sätze* |
|---|---|---|---|---|
| MVL | Guanidine hydrochloride 50–66 %<br>*Guanidinhydrochlorid 50–66 %* | Xn | R 22-36/38 | S 26-37/39 |
| MV2 | Sodium perchlorate 20–40 % + ethanol 35–55 %<br>*Natriumperchlorat 20–40 % + Ethanol 35–55 %* | Xn* | R 10-22 | S 13-16 |
| MV3 | Sodium perchlorate 5–20 % + ethanol 20–35 %<br>*Natriumperchlorat 5–20 % + Ethanol 20–35 %* | * | R 10 | S 16 |
| MV4 | Ethanol 55–75 %<br>*Ethanol 55–75 %* | F** | R 11 | S 7-16 |
| Carrier RNA Buffer | Guanidinium thiocyanate 30–60 %<br>*Guanidiniumthiocyanat 30–60 %* | Xn*<br>N* | R 20/21/22-32-52/53 | S 13-61 |
| Proteinase K | Proteinase K, lyophilized<br>*Proteinase K, lyophilisiert* | Xn | R 36/37/38-42 | S 22-24-26-36/37 |

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

** Hazard labeling not necessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

### Risk phrases

| | |
|---|---|
| R 10 | Flammable.<br>*Entzündlich.* |
| R 11 | Highly flammable.<br>*Leichtentzündlich.* |
| R 22 | Harmful by inhalation.<br>*Gesundheitsschädlich beim Verschlucken.* |
| R 20/21/22 | Harmful by inhalation, in contact with skin, and if swallowed.<br>*Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut.* |
| R 32 | Contact with acids liberates very toxic gas.<br>*Entwickelt bei Berührung mit Säure sehr giftige Gase.* |
| R 36/38 | Irritating to eyes and skin.<br>*Reizt die Augen und die Haut.* |
| R 36/37/38 | Irritating to eyes, respiratory system, and skin.<br>*Reizt die Augen, Atmungsorgane und die Haut.* |
| R 42 | May cause sensitization by inhalation.<br>*Sensibilisierung durch Einatmen möglich.* |
| R 42/43 | May cause sensitization by inhalation and skin contact<br>*Sensibilisierung durch Einatmen und Hautkontakt möglich.* |
| R 52/53 | Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.<br>*Schädlich für Wasserorganismen, kann in Gewässern längerfristig schädliche Wirkungen haben.* |

### Safety phrases

| | |
|---|---|
| S | Keep container tightly closed.<br>*Behälter dicht geschlossen halten.* |
| S 13 | Keep away from food, drink, and animal feedstuffs.<br>*Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.* |
| S 16 | Keep away from sources of ignition – No Smoking!<br>*Von Zündquellen fernhalten – Nicht rauchen.* |
| S 22 | Do not breathe dust.<br>*Staub nicht einatmen.* |
| S 24 | Avoid contact with the skin.<br>*Berührung mit der Haut vermeiden.* |
| S 26 | In case of contact with the eyes, rinse with plenty of water and seek medical advice.<br>*Bei Berührung mit den Augen gründlich mit Wasser abspülen und Arzt konsultieren.* |
| S 36/37 | Wear suitable protective clothing and gloves.<br>*Bei der Arbeit geeignete Schutzhandschuhe und Schutzkleidung tragen.* |
| S 37/39 | Wear suitable gloves and eye / face protection.<br>*Bei der Arbeit geeignete Schutzhandschuhe und Schutzbrille / Gesichtsschutz tragen.* |
| S 61 | Avoid release to the environment. Refer to special instructions / safety data sheet.<br>*Freisetzung in die Umwelt vermeiden. Besondere Anweisungen einholen / Sicherheitsdatenblatt zu Rate ziehen.* |

## 4.2 GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

*Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

| Component<br>*Inhalt* | Hazard contents<br>*Gefahrstoff* | GHS symbol<br>*GHS Symbol* | Hazard phrases<br>*H-Sätze* | Precaution phrases<br>*P-Sätze* |
|---|---|---|---|---|
| MVL | Guanidine hydrochloride 50–66 %<br>*Guanidinhydrochlorid 50–66 %* | Warning<br>*Achtung* | 302, 315, 319 | 280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313 |
| MV2 | Sodium perchlorate 20–40 % + ethanol 35–55 %<br>*Natriumperchlorat 20–40 % + Ethanol 35–55 %* | Danger<br>*Gefahr* | 226, 302 | 210, 233, 301+312, 330, 403+235 |
| MV3 | Sodium perchlorate 5–20 % + ethanol 20–35 %<br>*Natriumperchlorat 5–20 % + Ethanol 20–35 %* | Warning<br>*Achtung* | 226 | 210, 233, 403+235 |
| MV4 | Ethanol 55–75 %<br>*Ethanol 55–75 %* | Danger<br>*Gefahr* | 225 | 210, 233, 403+235 |
| Carrier RNA Buffer | Guanidinium thiocyanate 30–60 %<br>*Guanidiniumthiocyanat 30–60 %* | Warning<br>*Achtung* | 302, 412, EU031 | 260, 273, 301+312, 330 |
| Proteinase K | Proteinase K, lyophilized<br>*Proteinase K, lyophilisiert* | Danger<br>*Gefahr* | 315, 317, 319, 334, 335 | 261, 280, 302+352, 304+340, 305+351+338, 312, 333+313, 337+313, 342+311, 363, 403+233 |

**Hazard phrases**

| | |
|---|---|
| H 225 | Highly flammable liquid and vapour.<br>*Flüssigkeit und Dampf leicht entzündbar.* |
| H 226 | Flammable liquid and vapour.<br>*Flüssigkeit und Dampf entzündbar.* |
| H 302 | Harmful if swallowed.<br>*Gesundheitsschädlich bei Verschlucken.* |
| H 315 | Causes skin irritation.<br>*Verursacht Hautreizungen.* |
| H 317 | May cause an allergic skin reaction.<br>*Kann allergische Hautreaktionen verursachen.* |
| H 319 | Causes serious eye irritation.<br>*Verursacht schwere Augenreizung.* |

### Hazard phrases

| | |
|---|---|
| H 334 | May cause allergy or asthma symptoms or breathing difficulties if inhaled.<br>*Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.* |
| H 335 | May cause respiratory irritation.<br>*Kann die Atemwege reizen.* |
| H 412 | Harmful to aquatic life with long lasting effects.<br>*Schädlich für Wasserorganismen, mit langfristiger Wirkung.* |
| EUH 031 | Contact with acids liberates toxic gas.<br>*Entwickelt bei Berührung mit Säure giftige Gase.* |

### Precaution phrases

| | |
|---|---|
| P 210 | Keep away from heat/sparks/open flames/hot surfaces – No smoking.<br>*Von Hitze / Funken / offener Flamme / heißen Oberflächen fernhalten. Nicht rauchen* |
| P 233 | Keep container tightly closed.<br>*Behälter dicht verschlossen halten.* |
| P 260 | Do not breathe dust / fume / gas / mist / vapours / spray.<br>*Dampf nicht einatmen.* |
| P 261 | Avoid breathing dust.<br>*Einatmen von Staub vermeiden.* |
| P 273 | Avoid release to the environment.<br>*Freisetzung in die Umwelt vermeiden.* |
| P 280 | Wear protective gloves / eye protection.<br>*Schutzhandschuhe / Augenschutz tragen.* |
| P 301+312 | IF SWALLOWED: Call a POISON CENTER or doctor /physician if you feel unwell.<br>*Bei Verschlucken: Bei Unwohlsein Giftinformationszentrum oder Arzt anrufen.* |
| P 302+352 | IF ON SKIN: Wash with plenty of soap and water.<br>*Bei Kontakt mit der Haut: Mit viel Wasser und Seife waschen.* |
| P 304+341 | IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing.<br>*Bei Einatmen: Bei Atembeschwerden an die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.* |
| P 305+351+313 | IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing<br>*BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.* |
| P 330 | Rinse mouth.<br>*Mund ausspülen.* |
| P 333+313 | If skin irritation or a rash occurs: Get medical advice / attention.<br>*Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.* |
| P 342+311 | If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician.<br>*Bei Symptomen der Atemwege: Giftinformationszentrum oder Arzt anrufen.* |
| P 337+313 | Get medical advice / attention.<br>*Bei anhaltender Augenreizung: Ärztliche Rat einholen / ärztliche Hilfe hinzuziehen.* |
| P 363 | Wash contaminated clothing before reuse.<br>*Kontaminierte Kleidung vor erneutem Tragen waschen.* |
| P 403+235 | Store in a well ventilated place. Keep cool.<br>*Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.* |

For further information please see Material Safety Data Sheets ***(www.mn-net.com).***
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern* ***(www.mn-net.com)****.*

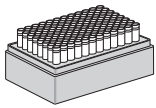
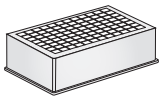
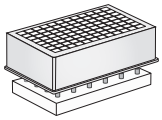
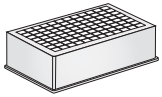
# 5 Protocol for the isolation of viral RNA/DNA from cell-free body fluids

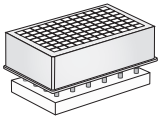
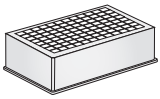
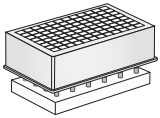
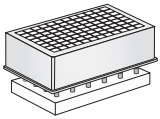
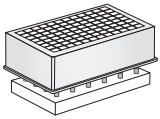
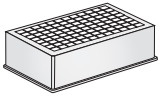
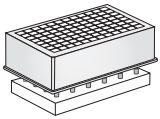
## Protocol-at-a-glance

- For hardware requirements refer to section 2.3.
- For detailed information on each step see page 17.

**Before starting the preparation:**

- Check if Proteinase K and Carrier RNA were prepared according to section 3.

| | | | |
|---|---|---|---|
| **1** | Lyse sample | **200 µL sample**<br>**10 µL Proteinase K**<br>**4 µL Carrier RNA**<br>**200 µL MVL**<br><br>**Mix**<br><br>**56 °C, 10 min** |  |
| **2** | Bind viral RNA/DNA to NucleoMag® V-Beads | **600 µL MV2**<br>**30 µL V-Beads** |  |
| | | **Mix by shaking for 5 min at RT**<br>*(Optional: Mix by pipetting up and down)* | ⟷ |
| | | **Remove supernatant after 2 min separation** |  |
| **3** | Wash with MV3 | **Remove Square-well Block from NucleoMag® SEP**<br><br>**500 µL MV3** |  |
| | | **Resuspend: Shake 1 min at RT** | ⟷ |

| | | | |
|---|---|---|---|
| | | **Remove supernatant after 2 min separation** |  |
| **4** | Wash with MV4 | **Remove Square-well Block from NucleoMag® SEP**<br><br>**500 µL MV4** |  |
| | | **Resuspend: Shake 1 min at RT** |  |
| | | **Remove supernatant after 2 min separation** |  |
| **5** | Wash with MV5 | **550 µL MV5**<br><br>**Incubate for 45–60 s**<br><br>*Note: Do not resuspend the beads in Buffer MV5!* |  |
| | | **Remove supernatant** |  |
| **6** | Elute RNA/DNA | **Remove Square-well Block from NucleoMag® SEP**<br><br>**50–100 µL MV6** |  |
| | | **Shake 5 min at 56 °C**<br>*(Optional: Mix by pipetting up and down)* |  |
| | | **Separate 2 min and transfer viral RNA/DNA into elution plate/tubes** |  |

## Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers. It is recommended using a Square-well Block for separation (see ordering information). Alternatively, isolation of viral RNA/DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

### 1 Lyse sample

Pre-dispense **10 µL Proteinase K** and **200 µL of sample** to a suitable reaction tube. Add **200 µL Buffer MVL** (with added **Carrier RNA**) to the reaction tube (If Carrier RNA is not premixed with the Buffer MVL, add 4 µL of the stock solution to the reaction tube). Mix well by repeated pipetting up and down and incubate at **56 °C** for **10 min**. Alternatively, lysis step can be performed in Tube Strips (see ordering information).

For higher convenience a premix of Proteinase K, Buffer MVL, and Carrier RNA can be prepared. This premix should be added to the sample immediately (within 15 min after preparation).

Following the lysis incubation, spin down to collect any sample from the lysis tube lids and transfer each lysate to the wells of a Square-well Block.

### 2 Bind viral RNA/DNA to magnetic beads

Add **30 µL resuspended V-Beads** and **600 µL Buffer MV2** to the lysed sample.

Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

NucleoMag® V-Beads and Buffer MV2 can be pre-mixed.

*Be sure to resuspend the NucleoMag® V-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.*

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP a magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

*Do not disturb the attracted beads while aspirating the supernatant.*

**3 MV3 wash**

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **500 µL Buffer MV3** and resuspend the beads by shaking until the beads are resuspended completely (1–3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

**4 MV4 wash**

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **500 µL Buffer MV4** and resuspend the beads by shaking until the beads are resuspended completely (1–3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

**5 MV5 wash**

Leave the Square-well Block on the NucleoMag® SEP magnetic separator. Gently add **550 µL Buffer MV5** to each well and incubate for **45–60 s** while the beads are still attracted to magnets. Then aspirate and discard the supernatant.

*Do not resuspend the beads in Buffer MV5. This step is to remove traces of ethanol and eliminates a drying step. Do not exceed incubation time of max. 1 min.*

**6 Elution**

Add desired volume of **Buffer MV6 (50–100 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5 min** at **56 °C.** Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5 min** at **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified viral RNA/DNA to either microtubes or Tube Strips (see ordering information).

# 6 Appendix

## 6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Poor yield/ low sensitivity | *Insufficient elution buffer volume*<br>• Beads pellet must be covered completely with elution buffer.<br><br>*Insufficient performance of elution buffer during elution step*<br>• Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps.<br><br>*Beads dried out*<br>• Do not let the beads dry as this might result in lower elution efficiencies.<br><br>*Aspiration of attracted bead pellet*<br>• Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate.<br><br>*Aspiration and loss of beads*<br>• Time for magnetic separation too short or aspiration speed too high. |
| Low purity/ low sensitivity | *Insufficient washing procedure*<br>• Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP.<br>• Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down. |
| Poor performance of RNA in downstream applications | *Carry-over of ethanol from wash buffers*<br>• Be sure to remove all of the ethanolic wash solution Buffer MV4, as residual ethanol interferes with downstream applications. |

| | |
|---|---|
| Poor performance of RNA in downstream applications *(continued)* | *Ethanol evaporation from wash buffers*<br>• Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs. |
| Carry-over of beads | *Time for magnetic separation too short*<br>• Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.<br>*Aspiration speed too high (elution step)*<br>• High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step. |

## 6.2 Ordering information

| Product | REF | Pack of |
|---|---|---|
| NucleoMag® 96 Virus | 744800.1<br>744800.4 | 1 x 96 preps<br>4 x 96 preps |
| NucleoMag® SEP | 744900 | 1 |
| Square-well Blocks | 740481<br>740481.24 | 4<br>24 |
| Self-adhering PE Foil | 740676 | 50 sheets |
| Rack of Tube Strips<br>(set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips) | 740477<br>740477.24 | 4 sets<br>24 sets |
| KingFisher® 96 Accessory Kit A Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® 96 Virus preps using King Fisher 96 platform | 744950 | 1 set |

Visit ***www.mn-net.com*** for more detailed product information.

## 6.3 Product use restriction/warranty

**NucleoMag® 96 Virus** kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

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DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN VITRO*-diagnostic use. Please pay attention to the package of the product. *IN VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

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